demonstrated that this mechanosensory response is tunable by varying the lever arm length of myosin-II heavy chain, showing that myosin-II is an active force sensor in this mechanosensory system. We now focus on how mechanical inputs mediated through myosin-II lead to changes in biochemical signaling pathways, specifically the cortexillin-I regulatory and spindle signaling pathways. Rac1A (a small GTPase), IQGAP1, and IQGAPA (GTPase effectors) can form complexes with cortexillin-I. In the absence of both IQGAP1 and IQGAPA, cortexillin-I does not localize normally to the cleavage furrow during cell division. However, IQGAPA, but not IQGAP1, is required for myosin-II mechanosensing. Kif12, a mitotic-kinesin-like protein in Dictyostelium cells, is part of the chromosomal passenger complex, including INCENP and Aurora kinase. Kif12 is also recruited to the cell cortex inside the micropipette in a myosin-II-dependent and/or IQGAPA-dependent manner during cell division. Thus, mitotic spindle signaling proteins are responsive to mechanical stress sensed by myosin-II. Overall, myosin-II is a key mechanical stress sensor and these mechanical inputs are fed back to the mitotic spindle signaling system.

836-Pos

Mechano-Chemical Feedbacks Play a Major Role in Regulating Actin Mesh Growth in Lamellipodial Protrusions

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During cell motion on a substratum, eukaryotic cells project sheet-like lamellipodia which contain a dynamically remodeling three-dimensional actin mesh. A number of regulatory proteins and subtle mechano-chemical couplings determine the lamellipodial protrusion dynamics. To study these processes, we constructed a microscopic physico-chemical computational model, which incorporates a number of fundamental reaction and diffusion processes, treated in a fully stochastic manner. Our work sheds light on the way lamellipodial protrusion dynamics is affected by the concentrations of actin and actin-binding proteins. In particular, we found that protrusion speed saturates at very high actin concentrations, where filament nucleation does not keep up with protrusion, resulting in sparse filamentous networks, and, consequently, high resistance forces on individual filaments. We also observed maxima in lamellipodial growth rates as a function of Arp2/3, a nucleating protein, and capping proteins. We provide detailed physical explanations behind these effects. In particular, our work supports the actin funneling hypothesis explanation of protrusion speed enhancement at low capping protein concentrations. Our computational results are in agreement with a number of related experiments. Overall, our work emphasizes that elongation and nucleation processes work highly cooperatively in determining the optimal protrusion speed for the actin mesh in lamellipodia.

837-Pos

In Silico Study of Formation and Collapse of T-Killer Cell Synapse Mediated by Receptor Recycling and Actin Network

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T-killer cells of the immune system eliminate virus-infected and tumorous cells through direct cell to cell interactions. Reorientation of the killing apparatus inside the T-killer cell to the interface with the target cell ensures specificity of the immune response. Several research works were reported to explain the mechanism of reorientation but the most adversary situation, when the cell's initial orientation is complete opposite to the desirable direction, always left skepticism toward the suggested mechanism. We have constructed a computational model that incorporate T-killer cell receptor dynamics and all the possible mechanical properties which involve not only intrinsic physiology of T-killer cell but also the synapse formation with target cell. The model studies show that the actin network nucleation and degradation is a crucial part in the T-killer cell synapse formation. Furthermore, the role of actin network provides a safety feature for the T-cell reorientation mechanisms by allowing T-cells to detach from the target cell when they are stranded in situations in which reorientation is not available. Our computational model also provides insights into the actin network near the T-cell synapse including retrograde flow development.

838-Pos

Intimacy Between Actin Network Flow and Turnover in the Lamella of Crawling Fragments

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Elucidating the dynamics of the actin cytoskeleton that generates the driving force for cell migration is fundamental to understanding the mechanisms of actin-based cell migration, which is important to various physiologically relevant processes including metastasis and angiogenesis.
In this study, based on the hypothesis that actin cytoskeleton in migrating cells is a spatiotemporally self-regulating structure, we aimed at elucidating the dynamic coupling between actin network flow and turnover by focusing on flow dynamics in the lamella of one of the simplest but elegant motility systems; crawling fragments derived from fish keratocytes. Using a combination of fluorescent speckle microscopy and particle imaging velocimetry, we have succeeded in quantitatively mapping the flow in the lamella of these simple motility systems where it was previously reported to be stationary. Moreover, by correlating network flow with turnover, we have demonstrated that whereas polymerization mediates network assembly at the front, surprisingly, network flow convergence modulates network disassembly toward the rear of the lamella, suggesting that flow and turnover are coupled during migration. Furthermore, we found that polymerization is not just limited to the usually reported narrow rim along the leading edge, but occurs over an extended ~8 ∈ 1/4m wide region at the posterior of the lamella. We suggest that this is necessary to maintain the structural integrity of the lamella for rapid cell migration, as in fish keratocytes. These results obtained using simple but remarkable motility systems present new interesting concepts about actin network dynamics during cell migration that will definitely have profound impact on cell migration research. We believe that this study will make a major contribution toward biophysical understanding of cell migration, and aid in the development of quantitative models for exploring the yet unknown mechanisms of the process.

839-Pos

Quantitative Analysis of Cell Edge Dynamics and Cell Shape in Non-Polarized Fish Epidermal Keratocytes

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Cell migration requires the coordination of several processes such as leading edge protrusion, adhesion formation and disassembly, and trailing edge retraction. The quantitative analysis concerning the correlation between these processes will be useful to understand the coordination mechanism. A major difficulty in the quantitative analysis stems from the complex morphology of migrating cells. To cope with this difficulty, we selected the non-polarized fish epidermal keratocytes as a simplified experimental system that includes basal migratory mechanisms. We acquired a mixture of non-polarized stationary keratocytes and polarized highly motile keratocytes by disaggregating the large epidermal sheets. The time-lapse micrographs of non-polarized keratocytes were used to analyze cell edge dynamics and cell peripheral shape. We adopted the protrusion and retraction rate and cell peripheral curvature as quantitative parameters. Non-polarized keratocytes did not exhibit net translocation, however, active protrusion and retraction were observed around the cell periphery. Protrusion rate was negatively correlated with the cell peripheral curvature. In contrast, retraction rate was positively correlated with the cell peripheral curvature. The plot of protrusion and retraction rates over the entire cell periphery showed that protrusion and retraction waves were traveling laterally in both directions along the cell periphery. The lateral traveling velocity of each wave was constant. The wave persistence varied from 10 s to 100 s. These results indicate that the cell has the positive feedback mechanism maintaining stable protrusion and retraction and that the rate of protrusion and retraction is related to the cell peripheral shape. Quantitative analysis together with theoretical and molecular biological studies will shed light on the mechanism of cell migration.

840-Pos

Mechanisms Underlying Protrusion-Retraction Waves at the Leading Edge of Migrating and Spreading Cells

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Motility is fundamental to many cell types and plays key roles in immune response, tissue development, and cancer metastasis. Recent studies of migrating mouse embryonic fibroblast cells revealed protrusion-retraction cycles and lateral waves at the leading edge [Giannone G, et al, Cell, (2004); Giannone G, et al, Cell (2007)]. Each cycle entails membrane protrusion powered by actin polymerization, interrupted every ~24 s by ~5 s partial retraction episodes attributed to myosin II proteins which pull back the growing lamellipodial actin network until the latter separates from the leading edge membrane. We developed a mathematical model and extended our experimental observations in order to quantitatively describe the mechanisms underlying this motility behavior. We find the retraction waves are caused by propagation of myosin powered tears between the lamellipodium and cell membrane. Once a tear is nucleated its